15 MASS SPECTROMETRY

15.1 Objectives

15.1.1 To familiarize the trainee with the theory and application of mass spectrometry in drug analysis

15.1.2 To familiarize the trainee with the MS instrumentation and software used in the laboratory

15.2 Modes of Instruction

15.2.1 Self-directed study through reading assignments

15.2.2 Presentations and demonstrations

15.2.3 Practical exercise

15.3 Reference

15.3.1 Moffat, A. C., editor. *Clarke's Isolation and Identification of Drugs*. London: The Pharmaceutical Press, 1986, pp. 251-263.

15.3.2 *Basic Training Program for Forensic Chemists*, U.S. Department of Justice, Drug Enforcement Administration, Office of Science and Technology, pp. 5-61 through 5-72.

15.3.3 Virginia Division of Forensic Science Drug Analysis Procedures Manual, Mass Spectrometry Section.

15.3.4 Saferstein, Richard, Ph.D. "Forensic Applications of Mass Spectrometry", in Saferstein, Richard, Ph.D., editor. *Forensic Science Handbook, Volume I*. Englewood Cliffs, N. J.: Prentice Hall, 1982, pp. 92-138.

15.3.5 Allen, A. C. et al. "The Cocaine Diastereomers", *Journal of Forensic Science*s, Vo., 26, No. 1, 1981.

15.3.6 Yinon, Jehuda. *Forensic Mass Spectrometry*. Boca Raton: CRC Press, Inc., 1987.

15.3.7 McLafferty, Fred W. and Venkataraghavan, Rengachari. *Mass Spectral Correlations, Second Edition.* Washington, D. C.: American Chemical Society, 1982.

15.3.8 McLafferty, F. W. *Interpretation of Mass Spectra, Second Edition*. Reading, MA: W. A. Benjamin, Inc., 1973.

15.3.9 Message, Gordon M. *Practical Aspects of Gas Chromatography/Mass Spectrometry.* New York: John Wiley & Sons, 1984.

15.3.10 Mills, Terry, III and Roberson, J. Conrad. *Instrumental Data for Drug Analysis, Second Edition*. Volumes 1-7, New York: Elsevier, 1987.

15.3.11 Computer-based NIST library of organic compounds (NIST98.l or higher)

15.3.12 Hewlett Packard and Agilent Technologies GC/MS instrument manuals

15.3.13 Hewlett Packard and Agilent Technologies computer-based tutorials

15.3.14 Silverstein, R. M. *et al*. *Spectrometric Identification of Organic Compounds* New York: John Wiley & Sons, 1991, pp. 3-41.

15.3.15 Watson, J. T. *Introduction to Mass Spectrometry*, 3rd ed., New York: Lippincott, 1997.

15.3.16 Steiner, R. "Mass Spectrometry Lecture", Virginia Division of Forensic Science, April 2000.

15.3.17 McFadden, W. *Techniques of Combined Gas Chromatography/Mass Spectrometry: Applications in Organic Analysis*, New York: Wiley-Interscience Publications, 1973.

15.3.18 Beynon, J. et al. *The Mass Spectra of Organic Molecules*, Amsterdam: Elsevier Publishing Co., 1968.

15.4 Assignments

15.4.1 Completion of required reading assignments (15.3.1, 15.3.3, 15.3.4, 15.3.14)

15.4.2 Study questions and practical exercise

15.5 Study Questions

15.5.1 What is mass spectrometry?

15.5.2 Describe the theory behind its use as an identification technique.

15.5.3 What types of information are obtained from a GC/MS?

15.5.4 Draw a schematic diagram of a GC/MS. What is the purpose of each component?

15.5.5 Define the following terms:

- Relative abundance
- Base peak
- Molecular ion
- Quasimolecular ion
- Parent/Precursor ion
- Daughter/Product ion
- Mass/charge ratio
- Mass spectrum
- Resolution
- Unit mass resolution
- Normalization
- DFTPP Normalization
- Carbonium ion
- Cleavage
- AMU
- Isobaric
- Radical
- Doubly charged ion
- Calibration compound
- Torr.
- Atmosphere
- Total Ion Current

15.5.6 What is a "metastable peak"? When and where does it occur?

15.5.7 What is the sensitivity of a GC/MS?

15.5.7.1 How do the various models of GC/MS systems in your lab compare with respect to sensitivity?

15.5.7.2 What factors determine this?

15.5.8 What is the difference between spectrometry and spectroscopy?

15.5.9 Describe any method considerations in using a MS detector instead of an FID detector for the GC.

15.5.9.1 Why is helium the preferred carrier gas?

15.5.9.2 What type of septa are required?

15.5.9.3 How does a Merlin Microseal work?

15.5.9.4 When would splitless injections be required? What changes are necessary in the method?

15.5.9.5 Explain the use of pulsed split and splitless injections.

15.5.10 Why can column bleed cause a problem in GC/MS and how is it corrected? Septum bleed?

15.5.11 How can non-volatile compounds be introduced into a mass spectrometer?

15.5.12 What things must an interface between a GC and a MS accomplish?

15.5.13 Describe how a jet separator works. How efficient is it?

15.5.14 Describe how a molecular membrane separator works. Why is it less suitable than a jet separator for drug analysis?

15.5.15 Explain and diagram the capillary direct method of sample transfer for the Agilent systems in the laboratory.

15.5.16 What is the most common mode of ionization?

15.5.17 Diagram the E.I. source for the Agilent 5973.

- Are the ions formed positive or negative?
- Do they have an even or odd number of electrons?
- What is the ionization efficiency of this technique?
- What governs the relative abundance of the ions formed?

15.5.18 What governs the number and energy of the electrons emitted by the filaments?

15.5.19 From what are the filaments made?

15.5.20 What is an "ionization appearance potential" curve?

15.5.20.1 What is the usual electron energy used in an E.I. source for complete ionization and why?

15.5.20.2 What effect does variation in this energy have on an ion abundance?

15.5.20.3 If a molecule is ionized with energy just at its appearance potential, what information may be obtained?

15.5.21 What vacuum conditions are necessary in the ionization source and the analyzing regions of a MS and why?

15.5.21.1 Describe how a rough pump works.

15.5.21.2 Describe how a diffusion pump works.

15.5.21.3 Describe how a turbomolecular pump works.

15.5.21.4 Is it necessary that the vacuum remain constant?

15.5.22 What temperature conditions must be maintained in the ion source?

15.5.23 Describe how the ions are accelerated once they are formed.

15.5.24 Explain how chemical ionization is performed.

15.5.24.1 What are its advantages/disadvantages with respect to electron ionization?

15.5.24.2 What is the number of fragment ions produced by this method dependent on?

15.5.24.3 Do the ions formed by this process have an even or odd number of electrons?

15.5.25 Describe how a quadrupole mass analyzer works.

15.5.25.1 What factors influence the practical limits of the quadrupole as a mass filter?

15.5.25.2 What determines whether an ion will have a stable trajectory through the quadrupoles?

15.5.25.3 Draw a graphical representation of ion stability for ramping DC and RF voltages in a quadrupole filter.

15.5.26 For the following detectors, describe the theory behind them as well as how they work:

- Time of flight
- Magnetic deflection mass analyzers
- Ion trap

15.5.27 Define mass resolution.

15.5.27.1 What does a resolution of 500 mean?

15.5.27.2 What is the resolution a function of?

15.5.27.3 Is the instrument in the laboratory a low, medium or high resolution instrument?

15.5.27.4 What resolution values are associated with these terms?

15.5.28 Describe how an electron multiplier works.

15.5.28.1 Why is it referred to as a continuous dynode?

15.5.28.2 What is the inner surface of the electron multiplier coated with?

15.5.28.3 What is the purpose of an x-ray shield, located between the quadrupole and the electron multiplier on the Agilent 5972?

15.5.28.4 Explain what a “high energy dynode” is and how it works.

15.5.29 What is a Faraday cup?

15.5.30 Why is the electron multiplier the detector of choice?

15.5.30.1 What are the limiting factors as to how well an electron multiplier can detect incoming ions?

15.5.31 Explain how the 10-peak and PBM library search routines work.

- How many peaks are stored in a library spectrum in each?
- How does the software decide which peaks to use?
- What makes a peak significant to each of these searches?
- What are the limitations of the computer library?

15.5.32 What reference spectra collections are available for your use?

- Do they consist of “normalized” data?
- Do they consist of DFTPP ion abundance calibrated data?
- Do they contain verified data?
- If not, are they still viable references for spectral comparisons?

15.5.33 List what the base peaks and molecular ions are for each of the following:

- Cocaine
- Heroin
- Phencyclidine

- LSD
- Methamphetamine

15.5.34 Can ephedrine and pseudoephedrine be distinguished by MS?

15.5.35 Can optical isomers and diastereomers be differentiated via MS?

15.5.36 Obtain a mass spectrum for cocaine and account for the major peaks in the spectrum.

15.5.37 Obtain literature mass spectra of the diastereoisomers of cocaine and discuss the differences.

15.5.38 List the isotopic abundances for each of the following elements: H, C, N, O, F, Si, P, S, Cl, Br, I

15.5.39 What is the nitrogen rule?

15.5.40 If a molecular formula has been determined, how can the number of rings and double bonds be determined?

15.5.41 What is the "index of hydrogen deficiency"?

15.5.42 What influences what bond sites will be ruptured to create molecular fragments?

15.5.43 Describe how fragmentation patterns are influenced by:

- Branched carbon atoms
- Double bonds
- Rings
- Hetero-atoms
- Carbonyl groups

15.5.44 What are the M+2 (or A+2) elements?

15.5.45 What percentage of intensity of a molecular ion is contributed to the M+1 peak by carbon atoms?

15.5.45.1 What is the formula for calculating the number of carbon atoms in a molecule?

15.5.45.2 How can the M+1 peak be used to determine the molecular weight?

15.5.46 What requirements are necessary for an ion to be considered a molecular ion?

15.5.46.1 What does increasing saturation and number of rings result in with respect to the abundance of a molecular ion?

15.5.46.2 What effect does chain branching have?

15.5.47 What is the most desirable characteristic of mass spectra of trimethylsilyl derivatives?

15.5.48 In what types of compounds is a molecular ion peak frequently not detectable?

15.5.49 In what types of compounds are molecular ion peaks most likely to occur?

15.5.50 What do the peaks occurring at higher mass numbers than the molecular ion represent?

15.5.51 Describe the isotope pattern for Cl and Br.

15.5.52 What ions can be associated with the following m/e ratios?

- 43
- 58
- 77
- 91

15.5.53 Define the term "logical neutral loss" and give examples.

15.5.54 What mass losses during fragmentation are highly unlikely?

15.5.55 Describe the term "rearrangement".

15.5.55.1 Describe a "gamma hydrogen (McLafferty) rearrangement" and show examples.

15.5.55.2 Describe an "adjacent hydrogen rearrangement" and show examples.

15.5.56 Define the following terms and describe how these terms relate mass spectrometry to chromatography?

- scan rate
- scan cycle time
- reset time
- a/d conversion rate
- spectral tilting
- Mass peak detect threshold
- GC peak detect threshold

15.5.57 Explain the terms "sequence file", "macro" and "data file" and how each of these relate to the various instruments in your laboratory.

15.5.58 Describe two different means of running a manual injection with the ChemStation software, and the results obtained.

15.5.59 What is the difference between "normalized" data and DFTPP ion abundance calibrated data?

- How is MS data usually normalized?
- DFTPP calibrated?
- Why are perfluorinated compounds used as calibration compounds?
- What does DFTPP stand for?

- PFTBA?
- Why is PFTBA preferred over DFTPP as an internal calibration standard?

15.5.60 What macros are used on the GC/MS in your laboratory and how do they work?

15.5.61 Explain sequencing and what its utility is.

15.5.62 Set up a sequence table on a Chemstation. Print out the result in "brief" format and describe what each field represents.

15.5.63 Describe the autotuning procedure, explaining what each part of the program accomplishes for each model MS in your laboratory.

15.5.64 Describe how to perform the following techniques:

- Headspace analysis
- Wet needle injection
- Quantitative analysis

15.5.65 What is SIM and what is it used for?

15.5.65.1 Can a MS be used for quantitation?

15.5.65.2 Under what conditions is this accomplished?

15.5.66 Describe the preventative maintenance schedule and the QA/QC procedures performed on the GC/MS.

15.5.66.1 Describe the tuning procedures employed when the primary operator is not present.

15.5.67 Describe the use of barcoding and how it relates to sample tracking.

15.5.68 Describe the conditions needed for using retention time data from GC/MS runs.

15.5.69 Describe the difference in general peak shape between a GC/MS run and a GC/FID run.

15.5.70 Describe the use of blanks on the GC/MS.

15.5.71 Describe the capabilities of the LC/MS including mass range.

15.5.72 Describe the various techniques of "Atmospheric Pressure Ionization" including:

15.5.72.1 Electrospray (ES)

15.5.72.2 Atmospheric pressure chemical ionization (APCI).

15.5.73 Explain as to a jury how a mass spectrometer operates.

15.6 Practical Exercises

15.6.1 Perform a standard spectra autotune on the GC/MS and describe what each value on the report represents. What types of parameter values may indicate a problem with the instrument?

15.6.2 Perform an autotune on the GC/MS and compare the results to the standard spectra autotune. Run the QA mix of drugs using both types of tunes and discuss the differences in spectra observed.

15.6.3 Compare the mass spectral data for ephedrine, pseudoephedrine, methamphetamine, and propoxyphene. What are the significant differences which make these spectra unique to their parent compound?

15.6.4 Compare the spectra of cocaine, heroin and methamphetamine. For the base peak, determine the percentage of the total ion current. How will this influence the certainty of the particular base peak?

15.6.5 Compare the mass spectra for LSD and LAMPA and indicate their differences.

15.6.6 Obtain an unknown spectrum from the TC. Using interpretive methods, give as much information about the unknown compound as possible.

15.6.7 Create two methods using the parameters listed below. Run a cocaine standard on each method and compare the results.

15.6.7.2 Method 1

- Oven temperature: 220 – 240 ºC @ 20 ºC / minute
- Scan Range: 400 – 14 amu
- a/d = 4

15.6.7.3 Method 2

- Oven temperature: 220 – 240 ºC @ 20 ºC / minute
- Scan Range: 400 – 14 amu
- a/d = 0

15.6.8 Change the background method so that the mass detect threshold is set to zero. Run the background and discuss the different possibilities for setting the thresholds in methods for drug analysis.

15.6.9 Obtain a sample of GHB from the training coordinator. Create the silyl derivative and analyze via GC/MS using the GHB procedure in the Controlled Substances Procedures Manual.

15.6.10 Develop a method to separate a cocaine/tetracaine mixture (0.01mg/mL) using splitless injection and pulsed splitless injection in full scan mode. Discuss any differences in chromatography and spectra.

15.6.11 Perform a headspace injection of a mixture of volatile solvents.

15.7 Modes of Evaluation

15.7.1 Written examination

15.7.2 Court exercise

♦ End